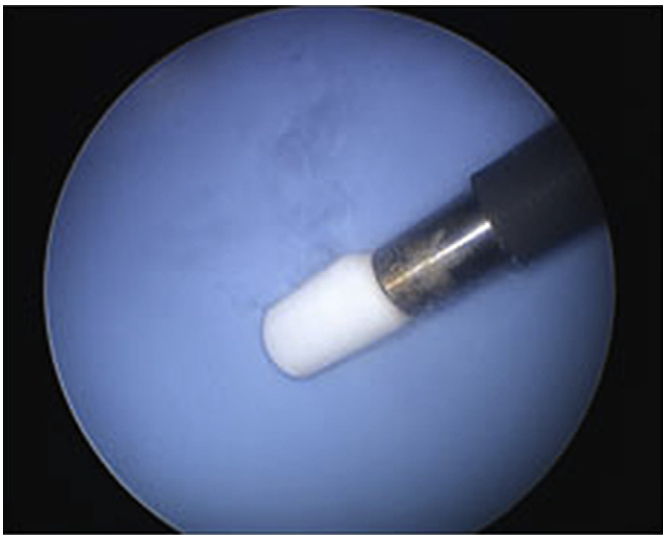
an engineered irrigant during endoscopic procedures designed to stabilize articular cartilage lesions.



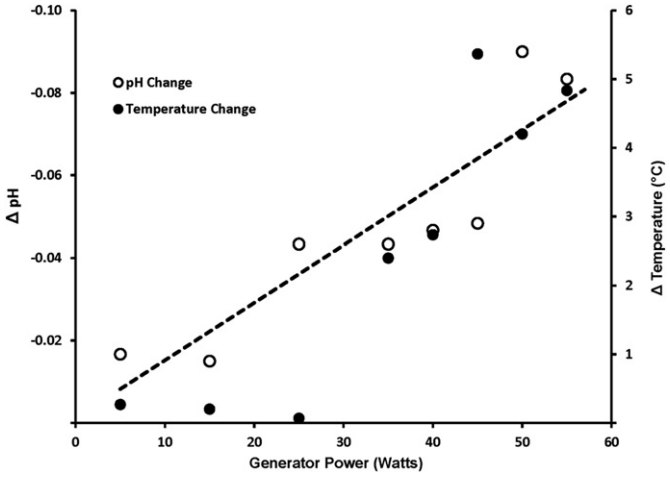
To determine if the engineered irrigant resembles the protic solvent generated by azurophilic granules, the resultant irrigant temperature and protonation potential were measured as a function of power input during device activation controlled for 5 second steady-state treatment conditions. Irrigant protonation potential was determined by measuring solution electrochemical potential relative to $[H^+]$ as a function of differential proton sequestration in the irrigant during device activation.

Results: Alternating current redox magnetohydrodynamics in saline solutions is represented in Figure 2.

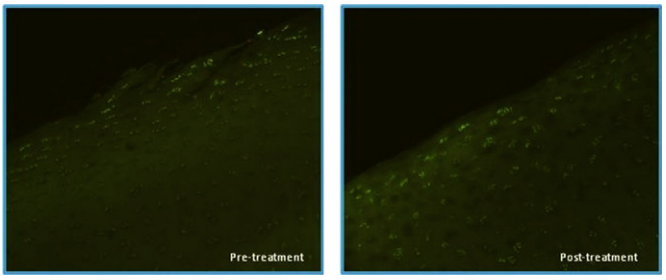
$$\alpha H_2O_{(l)} + \beta XCL \xleftrightarrow{\text{energy}} (\alpha-\beta)H_{2(g)} + \frac{(\alpha-\beta)}{2}O_{2(g)} + \beta HCl_{(aq)} + \beta XOH_{(aq)}$$

$$(\gamma-\delta)H_{2(g)} + \frac{(\gamma-\delta)}{2}O_{2(g)} + \delta HCl_{(aq)} + \delta XOH_{(aq)} \xleftrightarrow{\text{energy}} \gamma H_2O_{(l)} + \delta XCl_{(s)} + \Delta$$

As depicted in Figure 3, the protonation potential increased with direct correlation to power delivery ($p < 0.02$; $R^2 = 0.311$) and commensurate with a minimal change in irrigant temperature ($\sim$0-5^o C) above the baseline 20^o C, reflecting features characteristic of the protic solvent generated by the azurophilic degranulation of polymorphonuclear neutrophil granulocytes during the early phases of wound healing.



Conclusions: Because resection precision that eliminates both volumetric and functional over-resection is required before surgical lesion stabilization can be an effective aid to articular cartilage wound healing behaviors, polymorphonuclear neutrophil granulocyte function is a valuable therapeutic design resource. Protonation coupled conformational dynamics is an energy transduction processes that achieves nanometer resection precision through a guest chemical denaturization process below the isoelectric point of exposed damaged interstitial tissue matrices. Because of high proton motilities in water solutions, stoichiometric protonation is a very rapid charge redistribution process that leads to biopolymer disaggregation through molecular cleavage planes accessible due to normal tissue surface barrier losses and degenerate matrix properties characteristic of damage tissue sites. Figure 4 illustrates a representative integrated cell viability stain section image that demonstrates removing the bioburden of damaged tissue without iatrogenic over-resection in a human explant model of osteoarthritis by adapting alternating current redox magnetohydrodynamic technology for tissue rescue surgical procedures.



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EFFECT OF OXYGEN TENSION AND PH ON MITOCHONDRIAL FUNCTION IN HUMAN OSTEOARTHRITIC ARTICULAR CHONDROCYTES (HOAC)

J. Collins [1], R. Moots [2], P. Milner [1], P. Clegg [1]. [1] *Univ. of Liverpool, Neston, United Kingdom;* [2] *Univ. of Liverpool, University Hospital Aintree, Aintree, United Kingdom*

Purpose: Articular chondrocytes reside in a unique environment that is relatively hypoxic and acidic compared to other cells (Silver, 1975). Many *in vitro* models study chondrocyte biology at ambient conditions (e.g. 21% O_2, pH 7.2) which may be physiologically inappropriate. Additionally, oxygen and pH levels reduce further in joint disease (Gibson et al, 2008). Mitochondria are crucial cellular organelles and may be linked with osteoarthritis (Terkeltaub et al, 2002). This study investigated the effects of different oxygen tension and pH (in the absence or presence of the pro-inflammatory cytokine, IL-1β), on mitochondrial membrane potential, reactive oxygen species (ROS) levels and the glutathione antioxidant system

Methods: Primary human osteoarthritic articular chondrocytes were cultured in 3-D alginate beads in 0%, 1%, 5% or 21% oxygen for 48 hours at pH 7.2 or 6.2 in the absence or presence of IL-1β (10ng/ml). Mitochondrial membrane potential was assessed using the fluorescent dye JC-1. ROS levels were determined by dichlorofluorescein (DCF-DA). The reduced glutathione: oxidised glutathione (GSH: GSSG) ratio was analysed using the GSH/GSSG-Glo™ Assay (Promega).

Results: At pH 7.2, reductions from 5% O_2 (normoxia for cartilage *in vivo)* to 0% O_2 decreased cellular ROS levels by 53%. Acidosis (pH 6.2) increased cellular ROS by 40% (at 5% O_2). There was no difference in ROS levels between 5% and 21% O_2 levels at pH 6.2 or 7.2. Addition of IL-1β increased ROS levels in all conditions (except at 0% O_2 which was still lower than control levels). Hypoxia (0-1% O_2) decreased GSH: GSSG ratio, mainly by reducing GSH levels. GSH: GSSG was lowest in acidic (pH6.2) conditions in the presence of IL-1β, regardless of oxygen tension. Mitochondrial membrane potential depolarisation occurred in hypoxia (mirroring ROS levels) but also occurred in acidic conditions and in the presence of IL-1β.

Conclusions: These data demonstrate that oxygen tension and pH are important mediators of mitochondrial function and cellular antioxidant levels. The conditions that elicited optimal mitochondrial function were pH of 7.2 at5% O_2. Reductions in ROS levels, mitochondrial membrane potential and GSH: GSSG ratio were observed when oxygen tension and pH

were lowered, possibly mimicking the changes in disease where further hypoxia and acidosis are known features. Addition of IL-1β increased ROS levels in every condition, (except 0% O_2) possibly through inducing a respiratory burst. A decrease in GSH appears responsible for the decreases in the GSH: GSSG ratio seen in hypoxic and/or acidic conditions with or without IL-1β. This work demonstrates the importance of studying oxygen and pH on mitochondrial function in chondrocytes. The mechanisms behind this oxygen and pH-sensitivity require further characterisation.

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CHANGES IN THE INTEGRINS EXPRESSION ARE RELATED WITH THE LOSS OF EXTRACELLULAR MATRIX DURING THE OSTEOARTHRITIS PATHOGENESIS.

M. Almonte-Becerril, J.B. Kouri. *Centro de Investigación y de Estudios Avanzados del Inst. Politécnico Naciona (Cinvestav-IPN), México, D.F., Mexico*

Purpose: The aim of this study was to identify changes in the integrins expression from chondrocytes of the three zones of the cartilage and their possible role during the Osteoarthritis (OA) pathogenesis in an animal model.
Methods: The experimentally OA-induced model was accomplished by unilateral knee menisectomy and post-surgery training; normal rats were used as a control. Animals were sacrificed by CO2 overdose and right femoral condyles were removed and processed for electron microscopy (TEM) and Immunohistochemistry (IHC). Changes at ultrastructural level were observed by TEM at early stages of OA. In addition, the expression of integrins α2 and α5, as well as collagen I, collagen II and the caspase 3 active (C3A) were identified in cartilage at 1, 3, 5, 10, 20 and 45 training days (td) by IHC. At the same time, as a complementary method was identified the loss of proteoglycans (PG) through safranin O staining.
Results: Since early stages of OA, chondrocytes undergo changes at ultrastructural level as well as in their relationship with the extracellular matrix (ECM); these changes started in the superficial (SZ) and middle zones (MZ). In addition, integrins α2, α5 and collagen I were increased during the OA pathogenesis, showing their highest expression at 45 td in the SZ and MZ; however, collagen II and proteoglycans showed a tendency to decrease at late stages of OA. Furthermore the cell death was increased in late stages of OA mostly in the SZ and deep (DZ) zones.
Conclusions: The loss of ECM and death of chondrocytes are the central features during the OA pathogenesis. Our results suggest that in early stages of OA, chondrocytes changes present at ultrastructural and morphological levels, could be are related with their increased synthetic activity induced as a consequence of the mechanical damage. However, at late stages of OA, the loss of ECM (PG and collagen II) induces the increased expression of integrins; probably as a result of the inefficient ECM remodeling (synthesis of collagen I) and with the aim to avoid the cell death by the loss of survival signals. In addition, during OA pathogenesis, the presence of ECM fragments shows a proteolityc activity on the cartilage, inducing changes in the normal integrins signaling, which increase the OA severity.

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DIFFERENTIAL IMPACT OF GLUCOSAMINE SULFATE AND CUIVRAMINE ON THE IL-1β STIMULATED C-20/A4 CHONDROCYTE CELL LINE, *IN VITRO*.

F. Rousset [1], B. Lardy [1,2], L. Grange [1,2], F. Morel [1]. [1] *GREPI AGIM FRE3405, GRENOBLE, France;* [2] *Grenoble Hosp., Grenoble, France*

Purpose: The pathogenesis of primary osteoarthritis involves an imbalance between anabolic and catabolic pathways in chondrocytes where the reactive oxygen species (ROS) could play a central role. The expression of matrix metalloproteinases (MMPs), chondrocyte hypertrophy and apoptosis are the main features of the pathology. NADPH oxidase Nox4 is one of the 7 ROS generating Nox members expressed in human. It was shown to be expressed in human primary chondrocytes. Nox4 displays a constitutive NADPH oxidase activity that was previously reported to modulate proMMP1 expression and apoptosis of the C-20/A4 cell line. This pathway is down regulated by heme oxygenase-1 (HO-1). Glucosamine sulfate (GS), a basic structural element that composes cartilage proteoglycans is also a dietary supplement approved as a symptomatic slow-acting drug for osteoarthritis (SYSADOA). However, impact of GS on structural features of OA is relatively modest. To go further, Cuivramine (CA), a new dietary supplement containing GS (78.9%), copper sulfate (0.105%) and ginger root extract (5.26%) has been developed.
The aim of the study is to compare *in vitro* the effects of CA and GS on IL-1β stimulated C-20/A4 chondrocytes. Impact on ROS production (1), related MMPs expression (2), chondrocyte apoptosis (3) and mechanisms of action (4) has been investigated.
Methods: The antioxidant effects of GS and CA were investigated on HEK 293 TRex cells, a reproducible and reliable cell model to study Nox4. MMP expression and apoptosis were assessed on the human C-20/A4 chondrocyte cell line. The cells were pre-treated with 100 or 500μg/ml CA or GS during 96h. Then, they were stimulated by 2ng/ml IL-1β for 24h to assess the secretion of ADAMTS5, proMMP1 and proMMP13 or 96h to evaluate caspase 3 activation and viability. The expression of the antioxidant protein HO-1 was assessed by Western Blot.
Results: No direct antioxidant effects of CA and GS have been reported on the HEK 293 TRex cell line. However, the ROS production was significantly decreased (30%) after 96h pre-incubation with 500μg/ml CA. The proMMP1 expression was shown to be modulated by Nox4 derived ROS in C-20/A4 chondrocytes. The results have shown a significant decrease in proMMP1 expression (40%) in CA treated chondrocytes but not after GS treatment. This effect was dependant on ginger root and copper sulfate. Furthermore, ADAMTS5 expression was markedly decreased by GS and CA but not by ginger root and copper sulfate. On the other hand, there was no effect on proMMP13 expression. Moreover, results reported a significant decrease in the IL-1β induced caspase 3 activation in presence of GS and CA. Our data suggest that molecular mechanisms could involve HO-1.
Conclusions: In this study we provided experimental evidence *in vitro* that glucosamine sulfate decreases ADAMTS5 expression and apoptosis in the IL-1β stimulated C-20/A4 chondrocytes. In addition, ginger root and copper sulfate decrease the Nox4 regulated proMMP1 expression. These findings emphasize *in vitro* the potential beneficial effects of Cuivramine in osteoarthritis.

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THE EXPRESSIONS OF TYPEII COLLAGEN IS REGULATED BY PTEN IN HUMAN CHODROCYTES.

K. Iwasa, T. Nishiyama, S. Hayashi, T. Fujishiro, S. Hashimoto, K. Kawakita, S. Sakata, R. Kuroda, M. Kurosaka. *Kobe Univ. Graduate Sch., Kobe, Japan*

Purpose: Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) was identified as an important tumor suppressor gene. PTEN is second most frequently mutated gene in human cancer after p53. The function of PTEN is one of negative regulator of phosphoinositol-3-kinase (PI3K) signaling. PI3K pathway is critical for cell survival, differentiation and matrix synthesis. We have reported that apoptosis by shear stress in chondrocytes was dependent on p53, but the functions of PTEN in chondrocytes are still unknown. Therefore, we investigated the function of PTEN in chondrocytes.
Methods: Normal human chondrocytes were cultured on silicone chambers (STREX, Osaka, Japan), and stretched at 5% stress for 6 hours by pulse-motor-driven stretch machine (STREX). In order to inhibit the function of PTEN, PTEN siRNA was transfected to chondrocytes by lipofection method. After transfection of PTEN siRNA, chondrocytes were applied 5% or 10% stretch stress for 24 hours.
Further, chondrocytes were transfected with PTEN siRNA and treated with PI3K specific inhibitor (LY294002) and then, chondrocytes were applied at 5% stretch stress.
The expressions of PTEN and type II collagen (Col2a1) mRNA were analyzed by real-time PCR.
Results: The expression levels of PTEN were not changed by stretch stress, but the expression levels of Col2a1 were increased by 5% stretch stress and were decreased by 10% stretch stress (Figure1).